

3A assembly

1. Quantify the amount of plasmid found in the sample (ng/ μ L) and determine the quantity to add (you must have 500 ng in your mix).
2. For digestion, incubate to 37°C for 1 hour in the thermal cycler. Make 3 mixes with a final volume of 50 μ L as follows:

Mix	DNA	10X Buffer	Enzyme 1 (1 μ L)	Enzyme 2 (1 μ L)	Nuclease Free Water
Digestion Part A	Determine after quantification	5 μ L	Eco RI	Spe I	Complete the volume depending on your DNA concentration
Digestion Part B			Xba I	Pst I	
Digestion Plasmid			Eco RI	Pst I	

*Add enzymes at last

3. For inactivation, incubate to 80°C for 20 minutes in the thermocycler.
4. For ligation, incubate to 37°C for 1 hour in the thermal cycler (Final volume: 20 μ L).

	Volume (μ L)
Digested Part A	2
Digested Part B	2
Digested Plasmid	2
10X T4 Ligase Buffer	2
T4 DNA Ligase	1
Nuclease Free Water	11

*Make sure to add the ligase enzyme at last.

5. For inactivation, incubate to 80°C for 20 minutes in the thermocycler.
6. Transform with 2 μ L of the ligation mix.

For our 3A assembly, we used the next quantities according to our plasmid concentrations:

Part	Cuantification result (ng/mL)
1	18,26
2	19,86
3	16,53
4	19,86
5	19,93
6	20,36
7	19,03
Amp	28,8
Cm	39,83
Kan	31,3

Digestions

First assembly				Second assembly				Third assembly			
part 1	27.3822563	part 2	25.1762336	part 4	25.17623364	part 5	25.0878073	part 6	24.55795678	part 7	26.2743037
EcoRI-HF	1	XbaI	1	EcoRI-HF	1	XbaI	1	EcoRI-HF	1	XbaI	1
SpeI	1	PstI	1	SpeI	1	PstI	1	SpeI	1	PstI	1
10X NEBuffer	5	10X NEBuffer	5	10X NEBuffer	5	10X NEBuffer	5	10X NEBuffer	5	10X NEBuffer	5
H2O	15.6177437	H2O	17.8237664	H2O	17.82376636	H2O	17.9121927	H2O	18.44204322	H2O	16.7256963
AMP plasmid	17.3611111			AMP plasmid	17.3611111			AMP plasmid	17.3611111		
EcoRI-HF	1			EcoRI-HF	1			EcoRI-HF	1		
PstI	1			PstI	1			PstI	1		
10X NEBuffer 2.1	5			10X NEBuffer 2.1	5			10X NEBuffer 2.1	5		
H2O	25.6388889			H2O	25.6388889			H2O	25.6388889		

LIGACIONES

digested part 1	2			digested part 4	2			digested part 6	2		
digested part 2	2			digested part 5	2			digested part 7	2		
digested AMP plasmid	2			digested AMP plasmid	2			digested AMP plasmid	2		
10X T4 Ligase Buffer	2			10X T4 Ligase Buffer	2			10X T4 Ligase Buffer	2		
T4 DNA Ligase	1			T4 DNA Ligase	1			T4 DNA Ligase	1		
H2O	11			H2O	11			H2O	11		
	20				20				20		